

A New Variety of *Ranunculus japonicus* (Ranunculaceae) and Its Genetic Relationships to the Related Species of Sect. *Acris* in Japan

SYAMSUARDI¹, HIROSHI OKADA² and MAKOTO OGAWA³

¹ Universitas Andalas, Kampus Biologi, FMIPA, Unand, Limau Manis, Padang 25163, Indonesia;

² Botanical Gardens, Faculty of Science, Osaka City University, 2000 Kisaichi, Katano, Osaka 576-0004, Japan;

³ Tokushima Prefectural Museum, Bunka-no-Mori Park, Hachiman-cho, Tokushima 770-8074, Japan

The genetic relationships between the Higashi-Iyayama population and some other populations of *Ranunculus japonicus*, and two other closely related species, *R. yakushimensis* and *R. acris* var. *nipponicus*, were assessed morphologically, karyotypically, electrophoretically and by cross experiments. Plants of the Higashi-Iyayama population showed a leaf shape and the flowering time being similar to those of other *R. japonicus* populations but different from those of *R. acris* var. *nipponicus* and *R. yakushimensis* populations. On the other hand, plants of the Higashi-Iyayama population differed from those of other *R. japonicus* populations in both achene shape and plant size. These results suggest that plants of the Higashi-Iyayama population are not hybrids between *R. japonicus* and *R. acris* var. *nipponicus*, but rather are derivatives with some new characters from *R. japonicus*. The allozymic analysis and cross experiments show that the Higashi-Iyayama population is closely related to those of other *R. japonicus* and *R. yakushimensis*, but less closely to those of *R. acris* var. *nipponicus*.

Key words: allozyme variation, crossability, karyotype, new variety, pollen fertility, Ranunculaceae, *Ranunculus*

The sect. *Acris* Schur of the genus *Ranunculus* consists of ca. 150 species occurring over the world. These species are distributed mostly from warm climate zone to alpine zones; some species extend to subtropical, but rarely to tropical zones (Tamura 1967). There are four species of sect. *Acris* in Japan: *R. japonicus* Thunb., *R. acris* L. var. *nipponicus* Hara, *R. yakushimensis* (Makino) Masam. and *R. grandis* Honda (Hara & Kurosawa 1956, Tamura 1970). In Japan, *R. japonicus* is distributed widely, *R. yakushimensis* is endemic to Yakushima Island, whereas *R. acris* var. *nipponicus* and *R. grandis* are found in the rather isolated areas from each other (Hara & Kurosawa 1956, Ohwi & Kitagawa 1983).

During the fieldworks and the examination of herbarium specimens on *R. japonicus* and its related species in Japan, we encountered the unusual plants at

the Ochiai Pass, Higashi-Iyayama, Tokushima prefecture, Shikoku district. They seemed to be a small type of *R. japonicus* on the whole, but have the slightly beaked achenes. These plants might be hybrids between *R. acris* var. *nipponicus* with the strongly beaked achenes and the other species based on the achene morphology. But *R. acris* var. *nipponicus* is not found in Shikoku district so far.

In this study, we examined plants of the Higashi-Iyayama population of *R. japonicus* on their morphology, karyotype, pollen fertility, crossability and allozymic variations to clarify whether the plants were the interspecific hybrids or the dwarf derivatives with some new characters from *R. japonicus*.

Materials and Methods

In this study, the Higashi-Iyayama population is treated tentatively as an independent taxon (hereafter use "taxon" or "taxa" for species, variety and the Higashi-Iyayama population). The materials and the collection sites are listed in Table 1. Artificial F_1 hybrids between *R. japonicus* and *R. acris* var. *nipponicus* were made for the morphological comparisons. Samples were transplanted in the Botanical Gardens, Osaka City University. Voucher specimens are kept at the Tokushima Pref. Museum (TKPM) and the Botanical Gardens, Osaka City University.

Sixteen morphological characters were examined: leaf circumference (cm), leaf area (cm²), leaf length (cm), leaf width (cm), width of middle lobe (cm), depth of lobation (cm) and number of serration from rosette leaves (Fig. 1A), petal length (mm), petal width (mm), the number of ovary per flower and

the number of anther per flower. In addition, four discrete characters, i.e., the presence or absence of stolons, the transversal shape of stem, achene shape and the flowering time, were also recorded.

The flowering time was observed under the cultivated condition in the Botanical Gardens, Osaka City University.

For the estimation of pollen fertility, three to ten plants for each taxon were examined. Pollen was stained with 2 % lactophenol cotton blue solution (1:1:1:1 lactic acid, phenol, glycerin and H₂O), and 300-500 grains per anther were examined. Darkly stained and globose pollen grains were estimated as being fertile.

Somatic chromosomes were observed by the same methods as Okada & Tamura (1977).

To estimate crossability between plants of the

TABLE 1. Localities of populations of *Ranunculus japonicus*, the Higashi Iyayama population, *R. acris* var. *nipponicus* and *R. yakushimensis*. N: sample number.

Pop. Taxon/Locality no.	Altitude	Habitat	N
<i>Ranunculus japonicus</i>			
1 Yachonomori-1, Ishii-cho, Myozai-gun, Tokushima Pref.	20 m	open place in the park	20
2 Yachonomori-2, Ishii-cho, Myozai-gun, Tokushima Pref.	20 m	beside road	20
3 Yamagawa-cho, Oe-gun, Tokushima Pref.	350 m	ditch, beside road	24
4 Oshima, Anabuki-cho, Mima-gun, Tokushima Pref.	10 m	beside road	34
5 Matsuo, Handa-cho, Mima-gun, Tokushima Pref.	10 m	beside road	33
6 Nishitani, Mikano, Miyoshi-gun, Tokushima Pref.	10 m	beside road	23
Higashi-Iyayama population			
7 Ochiai Pass, Higashi-Iyayama, Miyoshi-gun, Tokushima Pref.	1550 m	grassland in mountain	40
8 Kanmine, Higashi-Iyayama, Miyoshi-gun, Tokushima Pref.	1600 m	grassland in mountain	20
<i>Ranunculus acris</i> var. <i>nipponicus</i>			
9 Mt. Tateyama, near Shoomyo river-1, Toyama Pref.	1000 m	mountain track	25
10 Mt. Tateyama, near Shoomyo river-2, Toyama Pref.	1000 m	mountain track	14
11 Iwayamatadani, Mt. Hakusan, Shiramine, Ishikawa-gun, Ishikawa Pref.	800 m	mountain track	29
<i>Ranunculus yakushimensis</i>			
12 Mt. Miyanoura-1, Kamiyaku-cho, Yakushima Isl., Kumage-gun, Kagoshima pref.	1600 m	mossy stone	18
13 Mt. Miyanoura-2, Yakucho, Yakushima Island, Kumage-gun, Kagoshima pref.	1500 m	peat bogs	14

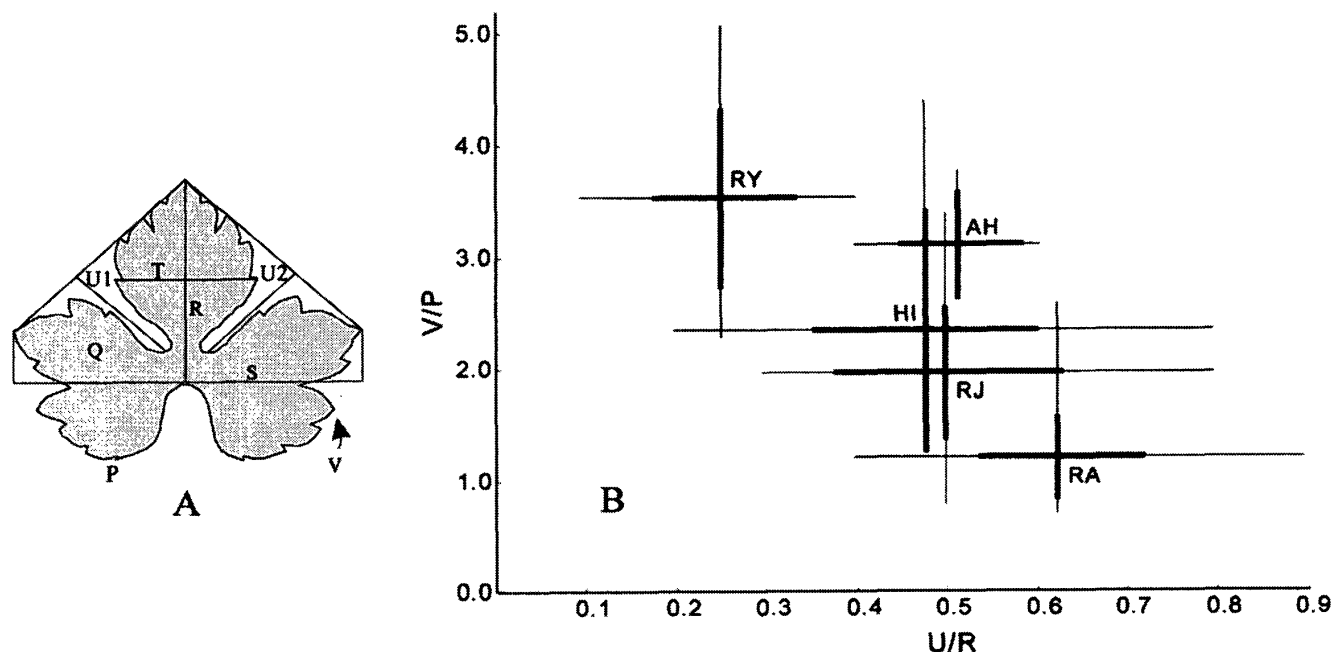


FIG. 1. Measurement of rosette leaves. A: circumference (P), area (Q), length (R), width (S), width of middle lobe (T), depth of lobation [$U = (U1+U2)/2$], number of serration (V). B: Diagram showing the difference between the Higashi-Iyayama population (HI) and some other populations of *Ranunculus japonicus* (RJ), *R. acris* var. *nipponicus* (RA), *R. yakushimensis* (RY) and the artificial F1 hybrid between *R. japonicus* and *R. acris* var. *nipponicus* (AH). Cross point: means. Thick line: standard deviation. Thin line: maximum and minimum values.

Higashi-Iyayama population and three taxa, 8 to 23 flowers were used. Crossing was carried out at one or two days later from the maturation of stigmatic area. Because the number of flowers for crossing test was restricted, non-reciprocal hybrid combination was made. *Ranunculus japonicus* was used as female parents for crosses between *R. japonicus* and other three taxa. For crosses between *R. acris* var. *nipponicus* and the Higashi-Iyayama population or *R. yakushimensis*, the former taxon was used as female parents. For crosses between the Higashi-Iyayama population and *R. yakushimensis*, the former taxon was used as female parents. After three weeks later from the crossing, fruits were harvested. The frequency of seed set was calculated from the ratio of fertile achenia per syncarp.

To analyze allozymic variation, two hundred milligrams of young leaves were homogenized in 2 ml of a grinding buffer made up of 0.1 M Tris-HCl (pH 7.5), 0.2 M sucrose, 2 % polyethylene glycol (PEG), 0.6 % polyvinylpyrrolidone (PVP), 0.001 M EDTA, 0.6

% mercapthoethanol and 0.1 % bovine serum albumin (BSA) (Stanton *et al.* 1997). Thirteen enzyme systems were resolved using Tris-HCl buffer system on a 7.5-11 % polyacrylamide gel electrophoresis system described by Shiraishi (1988): aspartate aminotransferase (AAT), esterase (EST), glutamate dehydrogenase (GDH), isocitric dehydrogenase (IDH), leucine aminopeptidase (LAP), malic enzyme (ME), malate dehydrogenase (MDH), menadione reductase (MNR), phosphoglucose isomerase (PGI), phosphoglucose mutase (PGM), 6-phosphoglucose dehydrogenase (6-PGD), shikimate dehydrogenase (SKDH) and superoxide dismutase (SOD). Eight enzyme systems (PGI, PGM, 6-PGD, IDH, SKDH, MDH, ME, and MNR) were resolved using Histidine buffer system on 12 % starch gel (Soltis *et al.* 1983). Slight modification was made by added 3 % sucrose into gel buffer.

Staining protocols followed Soltis *et al.* (1983). Except for EST, slight modification was made which gel was incubated in 0.2 M sodium acetate buffer (pH 5.0) before staining. The interpretation of coding

and banding patterns followed Shield *et al.* (1983) and Kephart (1990).

Genetic relationships were made using Nei's (1978) genetic identity (*I*) and distance (*D*), and the UPGMA clustering method based on Nei's genetic distance generated with GDA1-d16c (Lewis & Zaykin 2001).

Results

Statistic analysis on all twelve quantitative characters of external morphology showed that each taxon was distinguishable significantly from each other (Cruskall-Wallis One-way ANOVA, $P < 0.05$). Comparison of seven morphological characters of rosette leaves (leaf circumference, leaf area, leaf length, leaf width, width of middle lobe, depth of lobation and number of serration) and three characters of flowers (petal length, petal width and anther number per flower) indicated that plants of the Higashi-Iyayama population and *Ranunculus yakushimensis* were dwarf and clearly smaller than those of other populations of *R. japonicus* and *R. acris* var. *nipponicus* (Table 3). Ovary number of the Higashi-Iyayama population was not different significantly

from those of *R. japonicus* (Mann-Whitney U test, $P > 0.05$). In the qualitative characters, *R. yakushimensis* has overground stolon and solid stem, but other three taxa as well as the artificial F_1 hybrid of *R. japonicus* and *R. acris* var. *nipponicus* have no stolon and their stems are hollow (Table 2). The slightly beaked achenia of plants from the Higashi-Iyayama population seem to be intermediate between the strongly beaked achenia of *R. acris* var. *nipponicus* and the beakless achenia of *R. japonicus* (Fig. 2A), *R. yakushimensis* and the F_1 hybrids between *R. japonicus* and *R. acris* var. *nipponicus*. Flowering time of *R. yakushimensis* and *R. acris* var. *nipponicus* overlapped largely, while flowering time of *R. japonicus* was almost the same as that of the Higashi-Iyayama population and of the F_1 hybrids between *R. japonicus* and *R. acris* var. *nipponicus*.

Pollen fertility of the Higashi-Iyayama population (45 %) was significantly lower than those of *Ranunculus japonicus* (92%), *R. acris* var. *nipponicus* (85%), *R. yakushimensis* (90%), as well as the F_1 hybrids between *R. japonicus* and *R. acris* var. *nipponicus* (90 %) (Mann-Whitney U test, $P < 0.05$).

Somatic chromosome numbers of all *Ranunculus japonicus*, *R. yakushimensis*, *R. acris* var. *nipponicus*

TABLE 2. Diagnostic characters of *Ranunculus japonicus*, the Higashi-Iyayama population, *R. acris* var. *nipponicus*, *R. yakushimensis* and *R. grandis*.

Character	<i>R. japonicus</i>	Higashi- Iyayama population	<i>R. acris</i> var. <i>nipponicus</i>	<i>R. yakushimensis</i>	<i>R. grandis</i>
Chromosome number (2n)	14	14	14	14	28, 42
Achene shape	beakless	somewhat beaked	strictly beaked	beakless	beakless
Overground stolon	absent	absent	absent	present	absent
Subterranean stolon	absent	absent	absent	absent	present
Stem	hollow	hollow	hollow	solid	hollow
Radical leaf	thin, shallowly incised	thin, shallowly incised	thin, much divided	thick, lobed	thin, shallowly incised
Flowering time*	April-middle May	early April-middle May	late July-early August	early-middle August	middle-late May

* data from the observations under the cultivated condition in the Botanical Gardens, Osaka City University except for *R. grandis*.

TABLE 3. Morphological characters in *Ranunculus japonicus*, the Higashi-Iyayama population, *R. acris* var. *nipponicus* and the artificial F₁ hybrids between *R. japonicus* and *R. acris* var. *nipponicus*. All of the quantitative characters were significantly different among taxa (Kruskal-Wallis One-way ANOVA, $P < 0.05$). The number of plants examined is given in parenthesis. SD: standard deviation.

Characters	<i>R. japonicus</i> Mean \pm SD (N)	Higashi-Iyayama population Mean \pm SD (N)	<i>R. acris</i> var. <i>nipponicus</i> Mean \pm SD (N)	Artificial F ₁ hybrids Mean \pm SD (N)
Leaf circumference (cm)	27.5 \pm 15.6 (154)	11.4 \pm 5.0 (53)	32.7 \pm 10.0 (76)	11.6 \pm 1.0 (4)
Leaf area (cm ²)	19.3 \pm 9.7 (154)	4.4 \pm 2.6 (53)	54.2 \pm 40.6 (76)	4.2 \pm 1.1 (4)
Leaf length (cm)	3.9 \pm 4.1 (154)	1.8 \pm 0.5 (53)	4.0 \pm 1.0 (76)	2.1 \pm 0.4 (4)
Leaf width (cm)	6.3 \pm 2.0 (154)	2.7 \pm 1.0 (53)	6.2 \pm 1.4 (76)	3.2 \pm 0.3 (4)
Width of middle lobe (cm)	2.7 \pm 0.8 (154)	1.1 \pm 0.4 (53)	2.6 \pm 0.8 (76)	1.5 \pm 0.2 (4)
Depth of lobation (cm)	3.9 \pm 1.1 (154)	1.8 \pm 0.5 (53)	4.0 \pm 1.0 (76)	2.1 \pm 0.4 (4)
Number of serration	53.3 \pm 28.1 (154)	23.7 \pm 7.4 (53)	37.2 \pm 10.4 (76)	35.7 \pm 4.9 (4)
Petal length (mm)	11.3 \pm 2.8 (21)	8.1 \pm 0.8 (19)	9.9 \pm 1.6 (27)	8.6 \pm 0.3 (3)
Petal width (mm)	8.7 \pm 1.2 (27)	4.1 \pm 0.6 (19)	8.5 \pm 1.6 (21)	6.9 \pm 0.3 (3)
Ovary no. per flower	27.9 \pm 0.4 (21)	27.5 \pm 0.6 (24)	33.5 \pm 0.8 (22)	26.3 \pm 1.1 (3)
Anther no. per flower	58.0 \pm 8.7 (21)	37.8 \pm 1.1 (24)	85.0 \pm 13.6 (22)	56.7 \pm 4.9 (3)
Pollen fertility (%)	92.0 \pm 9.0 (10)	45.0 \pm 24.0 (6)	85.0 \pm 4.0 (6)	90.0 \pm 2.0 (3)

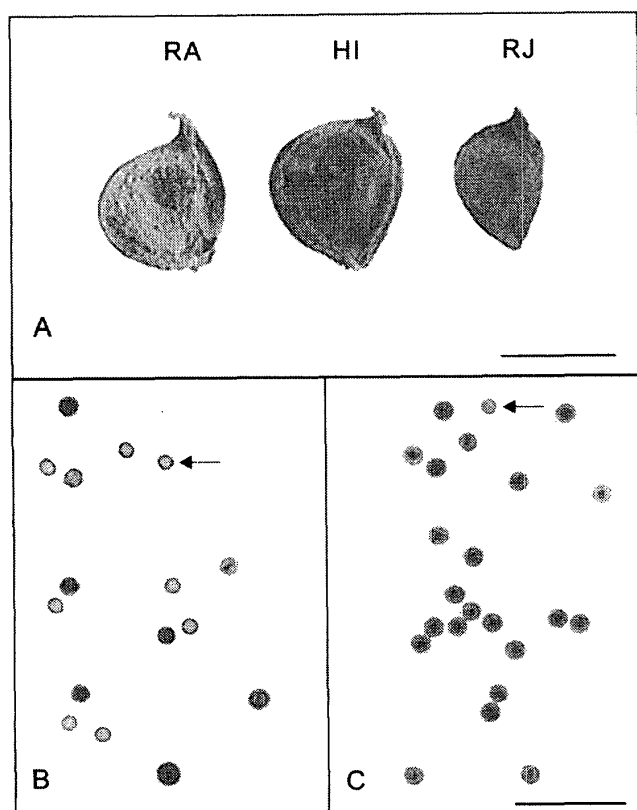


FIG. 2. Achene shape (A) of *Ranunculus acris* var. *nipponicus* (RA), the Higashi-Iyayama population (HI) and *R. japonicus* (RJ). Many sterile pollen grains are produced in the Higashi-Iyayama population (B), and almost all pollen grains are fertile in *R. japonicus* (C). Arrows in Figs. 2B and C indicate sterile pollen grains. Bar represents 1.0 mm (A) and 150 μ m (B, C)

and the Higashi-Iyayama population were $2n = 2x = 14$, and their karyotypes were almost similar to each other (Figs. 3, 4).

Seed set varied by the cross combination of taxa. The highest seed set (81%) was observed in the case of cross between *Ranunculus japonicus* and the Higashi-Iyayama population, followed the cross between *R. japonicus* and *R. yakushimensis* (72 %). Seed sets of the cross between *R. acris* var. *nipponicus* and *R. yakushimensis* or the Higashi-Iyayama population were rather low (Fig. 5). The lowest value was observed in the case of cross between *R. japonicus* and *R. acris* var. *nipponicus* (46 %).

Of the thirteen enzyme systems examined, six (AAT, EST, MNR, PGI, PGM, and 6-PGD) could be constantly resolved and scored for 11 loci (*Aat*, *Est-1*, *Est-2*, *Mnr*, *Pgi-1*, *Pgi-2*, *Pgm-1*, *Pgm-2*, *Pgm-3*, *6-Pgd-1*, and *6-Pgd-2*), while the remaining enzymes could not be used for the present study due to the inconsistent staining or poor resolution.

Ranunculus japonicus had twelve unique alleles. Seven alleles of *R. acris* var. *nipponicus*, however, were not detected in the *R. japonicus* (Table 4). Three alleles of the Higashi-Iyayama population were not detected in *R. acris* var. *nipponicus*. In contrast, nine

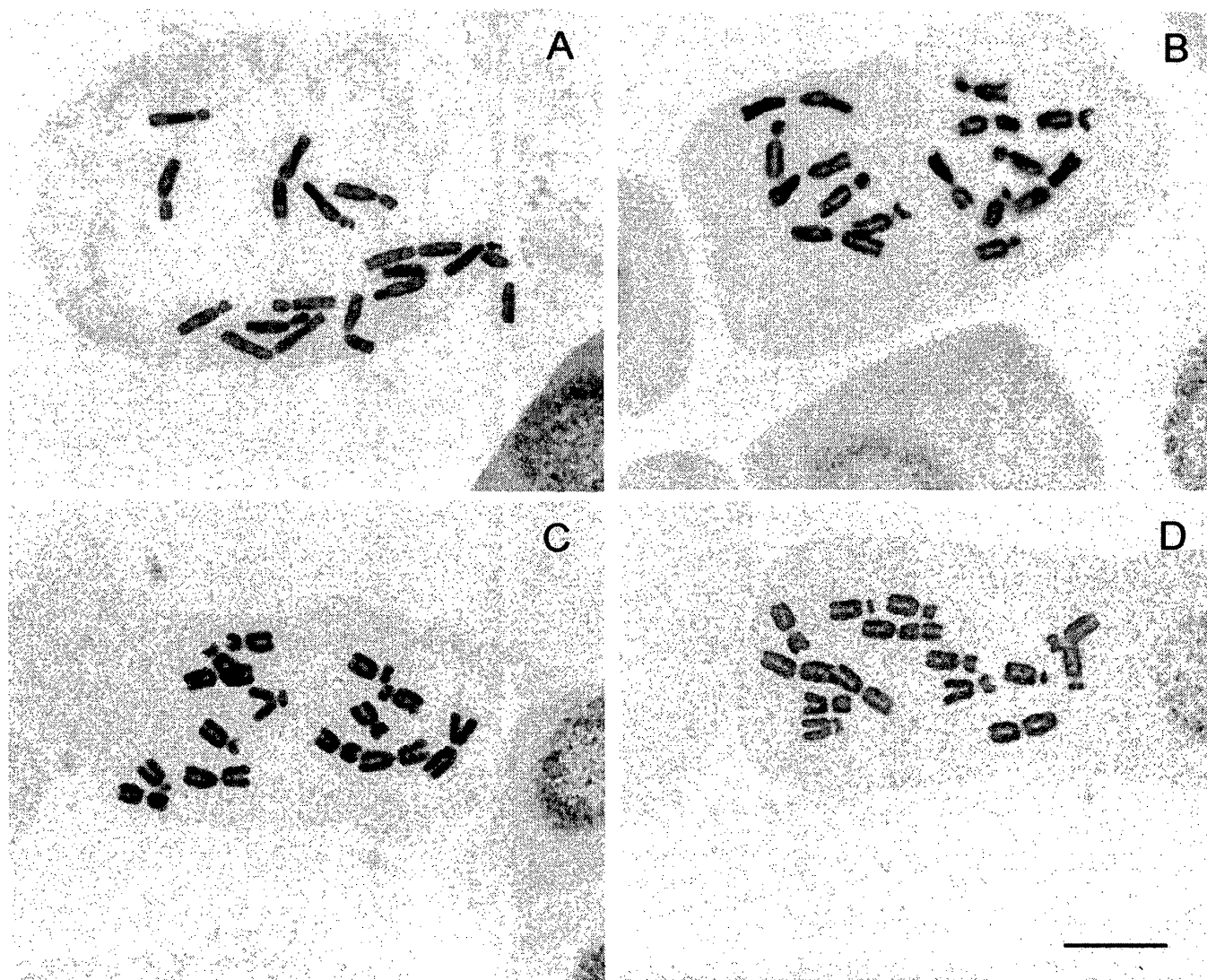


FIG. 3. Photomicrograph of somatic metaphase chromosomes of *Ranunculus japonicus* (A), *R. acris* var. *nipponicus* (B), plants from the Higashi-Iyayama population (C) and *R. yakushimensis* (D). Bar=10 μ m.

alleles of *R. acris* var. *nipponicus* were not detected in the Higashi-Iyayama population. All alleles found in the Higashi-Iyayama population and *R. yakushimensis* were found as subsets in those of *R. japonicus*. Further, two alleles, i.e., *Est-2^a* and *Mnr^f*, were observed very frequently in *R. acris* var. *nipponicus* (0.566 and 0.988, respectively) but rare in other three taxa (0.112 to 0.277, and 0.152 to 0.472, respectively). The allele *Est-2^c* was detected frequently in *R. japonicus* (0.812), the Higashi-Iyayama population (0.849) and *R. yakushimensis* (1.000), but very rare in *R. acris* var. *nipponicus* (0.036).

Nei's genetic identities were different by the

combination of taxa (Table 5). The genetic identity between the Higashi-Iyayama population and *Ranunculus japonicus* ($I = 0.944$) and the Higashi-Iyayama population and *R. yakushimensis* ($I = 0.938$) were significantly higher than those of the Higashi-Iyayama population and *R. acris* var. *nipponicus* ($I = 0.863$) (Mann-Whitney U test, $P < 0.05$). The cluster analysis indicates that the populations of *R. acris* var. *nipponicus* were distantly related to the other populations of *R. japonicus*, the Higashi-Iyayama population and *R. yakushimensis* (Fig. 6).



FIG. 4. Somatic metaphase chromosomes of *Ranunculus japonicus* (A), *R. acris* var. *nipponicus* (B), plants from the Higashi-Iyayama population (C) and *R. yakushimensis* (D). Bar=5 μ m.

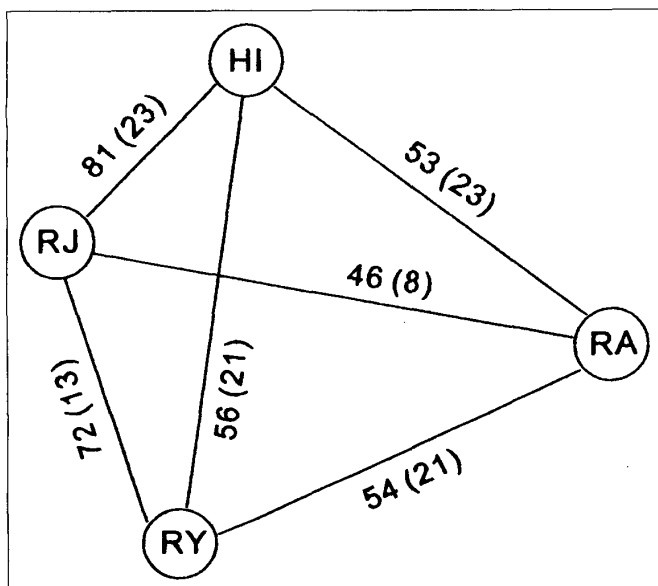


FIG. 5. Chart of seed set (%) obtained from crossing tests between *Ranunculus japonicus* (RJ), the Higashi-Iyayama population (HI), *R. acris* var. *nipponicus* (RA) and *R. yakushimensis* (RY). Numbers of plants examined are given in parenthesis.

Discussion

Based on the intermediacy of achene shape (Fig. 2A) and the low pollen fertility (Table 3, Fig. 2B), we assumed that plants of the Higashi-Iyayama popula-

tion were natural hybrids between *Ranunculus acris* var. *nipponicus* and one of the other taxa, such as *R. japonicus*, *R. yakushimensis* or *R. grandis*. Therefore, as the first step, we attempted to identify the putative parents of plants of the Higashi-Iyayama population. It seems that *R. acris* var. *nipponicus* and *R. japonicus* is more possible as parent species of the Higashi-Iyayama population than *R. yakushimensis* or *R. grandis* from the following information. The Higashi-Iyayama population differed from *R. yakushimensis* in the characters of overground stolon (absent vs. present), stem (hollow vs. solid) and radical leaf (thin vs. thick), and from *R. grandis* in ploidy level (2x vs. 4x or 6x) and in subterranean stolon (absent vs. present) (Table 2).

As the second step, we compared the external morphology of plants among the Higashi-Iyayama population, *R. japonicus*, *R. acris* var. *nipponicus*, and the artificial F₁ hybrids between *R. japonicus* and *R. acris* var. *nipponicus* (Table 3, Figs 1, 2). Plants of the Higashi-Iyayama population differed from F₁ hybrids between *R. japonicus* and *R. acris* var. *nipponicus* in almost all characters examined, i.e., number of serration of the leaf, achene shape and number of anther per flower (Table 3, Fig. 1B). These results showed that plants from the Higashi-Iyayama population were not the F₁ hybrids between *R. japonicus* and *R. acris* var. *nipponicus*. *Ranunculus japonicus* is reproductively isolated from *R. acris* var. *nipponicus* (Table 2) due to non-overlapping flowering time. Furthermore, *R. japonicus* is geographically isolated from *R. acris* var. *nipponicus* in Shikoku district. Therefore, it is impossible to form hybrids between both taxa in nature.

The plants from the Higashi-Iyayama population have the similar but smaller leaf shape of these from *Ranunculus japonicus* (Fig. 1, Table 3) and occur at 1500 m alt. (which is extremely high altitude for *R. japonicus*), where growing period is limited. Thus the plants from the Higashi-Iyayama population might have been derived by the neotenic change on the sizes of the plant body and flower. Its achene shape

TABLE 4. Mean allele frequency for polymorphic loci in *Ranunculus japonicus*, the Higashi-Iyayama population, *R. acris* var. *nipponicus* and *R. yakushimensis*. N = number of populations. Number of plants examined is given in parenthesis.

Locus	Allele	<i>R. japonicus</i> N = 6 (154)	Higashi-Iyayama population N = 2 (53)	<i>R. acris</i> var. <i>nipponicus</i> N = 3 (76)	<i>R. yakushimensis</i> N = 2 (32)
Aat	a	0.319	0.088	0.023	0.417
	b	0.620	0.913	0.881	0.584
	c	0.061	-	0.097	-
Est-1	a	0.007	0.121	-	-
	b	0.100	0.038	0.173	-
	c	0.880	0.841	0.827	1.000
	d	0.013	-	-	-
Est-2	a	0.112	0.152	0.566	-
	b	-	-	0.399	-
	c	0.812	0.849	0.036	1.000
	d	0.076	-	-	-
Mnr	a	0.183	-	0.012	0.201
	b	0.253	0.528	-	-
	c	0.047	-	-	-
	d	0.228	-	-	0.415
	e	0.012	-	-	-
	f	0.277	0.472	0.988	0.385
6Pgd-1	a	0.991	1.000	1.000	1.000
	b	0.010	-	-	-
6Pgd-2	a	-	-	0.013	-
	b	0.998	1.000	0.987	1.000
	c	0.003	-	-	-
Pgi-1	a	-	-	0.420	-
	b	0.973	1.000	0.554	1.000
	c	0.027	-	-	-
	d	-	-	0.027	-
Pgi-2	a	-	-	0.167	-
	b	0.976	1.000	0.780	1.000
	c	0.024	-	-	-
	d	-	-	0.053	-
Pgm-1	a	0.070	0.025	-	-
	b	0.923	0.975	1.000	1.000
	c	0.007	-	-	-
Pgm-2	a	0.021	-	-	-
	b	0.927	1.000	1.000	1.000
	c	0.052	-	-	-
Pgm-3	a	0.021	-	-	-
	b	0.979	1.000	0.988	1.000
	c	-	-	0.012	-

TABLE 5. Nei's coefficient of genetic identities among *Ranunculus japonicus*, the Higashi Iyayama population, *R. acris* var. *nipponicus* and *R. yakushimensis*. Mean genetic identities with the same superscript letter are not significantly different ($P < 0.05$) in Mann-Whitney U test. The range of genetic identity values is given in parenthesis.

Taxa	<i>R. japonicus</i>	Higashi-Iyayama population	<i>R. acris</i> var. <i>nipponicus</i>
Higashi-Iyayama population	0.944 ^b (0.913-0.984)	–	
<i>R. acris</i> var. <i>nipponicus</i>	0.853 ^c (0.754-0.904)	0.863 ^c (0.796-0.921)	–
<i>R. yakushimensis</i>	0.975 ^a (0.917-0.992)	0.938 ^b (0.897-0.975)	0.848 ^c (0.806-0.884)

might be derived by simple mutations. At present, it remains unclear why the plants of the Higashi-Iyayama population showed low pollen fertility.

Based on the above evidences, we conclude that plants of the Higashi-Iyayama population are not hybrids, but rather derivatives with some new characters from *R. japonicus*. Infra-specific taxa can be recognized and named if they exhibit clear delimitation from other taxa by non overlapping discontinuity in one or more characters, and have a geographical basis (Brunel & Whitkus 1999). We accepted their concept to evaluate the taxonomic status of the plants of the Higashi-Iyayama population and describe it as a new variety of *R. japonicus*.

As the third step, we analyzed the genetic relationships among diploid taxa, namely, *Ranunculus japonicus*, *R. yakushimensis*, *R. acris* var. *nipponicus* and the Higashi-Iyayama population using elec-

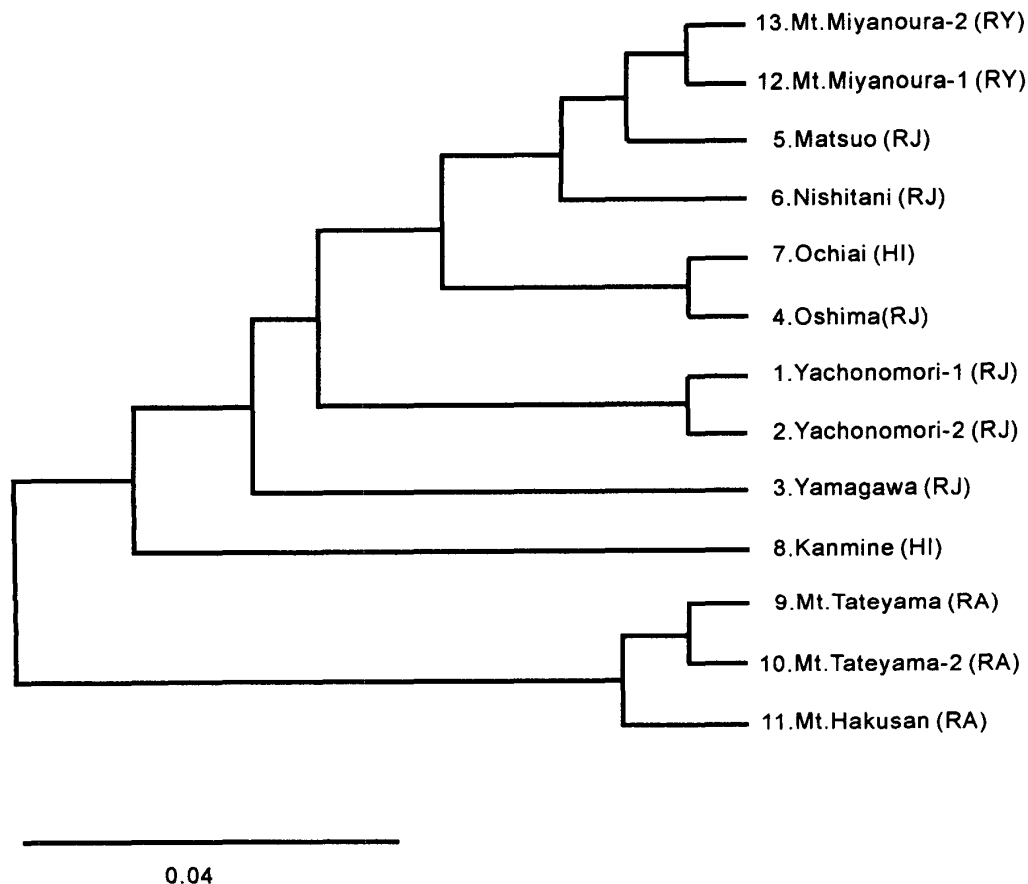


FIG. 6. UPGMA clustering analysis derived from Nei's genetic distance coefficients of all pairwise comparisons of populations among *Ranunculus japonicus* (RJ), the Higashi-Iyayama population (HI), *R. acris* var. *nipponicus* (RA) and *R. yakushimensis* (RY).

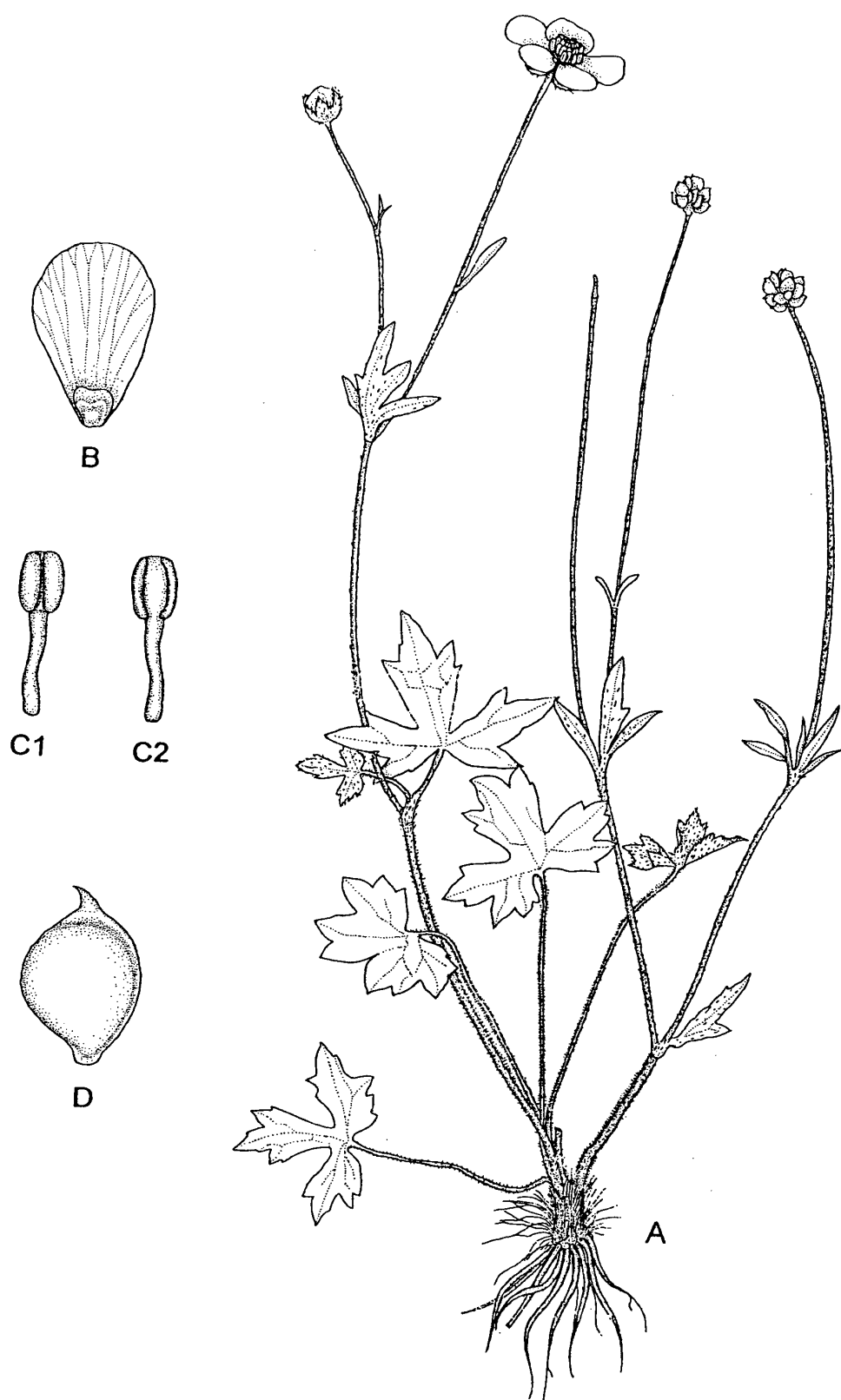


FIG. 7. *Ranunculus japonicus* var. *rostratus* (= the Higashi-Iyayama populations). A. Habit with rosette and cauline leaves. $\times 1.0$. B. Petal with flabellate nectary scale. $\times 4.0$. C1. Stamen, front view. $\times 6.5$. C2. Stamen, back view. $\times 6.5$. D. Beaked achene. $\times 8.0$. All figures are drawn from *M. Ogawa 4252* (TKPM).

trophoretical analysis. Nei's genetic identities indicated that the Higashi-Iyayama population related more closely to *R. japonicus* ($I = 0.944$) or to *R. yakushimensis* ($I = 0.938$), than to *R. acris* var. *nipponicus* ($I = 0.863$) (Table 5, Fig. 6). The former two values are slightly higher than those in conspecific species pairs (Gottlieb 1977, Gottlieb *et al.* 1985, Ranker & Scnoble 1986, Crawford & Whitkus 1988), while the latter value almost corresponds to the congeneric species pair. These results suggest that very little genetic divergence has been occurred among the Higashi-Iyayama population, *R. japonicus* and *R. yakushimensis*. And all those alleles of plants in the Higashi-Iyayama population were a subset of those found in *R. japonicus* and *R. yakushimensis*. Although *R. yakushimensis* shows the close genetic similarities with the Higashi-Iyayama population, it is very difficult to consider the direct phylogenetic relationship between *R. yakushimensis* and the Higashi-Iyayama population, because the former is endemic to the montane zone of Yakushima Island, while the latter is endemic to the montane area of Shikoku district.

Taxonomic treatment of the Higashi-Iyayama population

***Ranunculus japonicus* Thunb. var. *rostratus* Syamsuardi, H. Okada et M. Ogawa, var. nov.** (Fig 2, 7).

Haec varietas nova a typo foliis et floribusque minusculis et fructibus rostratis diversa.

Type: JAPAN, Shikoku Isl., Tokushima Pref., Miyoshi-gun, Higashi-Iyayama-son (village), Ochiai-pass, June 26, 1991, *M. Ogawa 4252* (TKPM).

Small, perennial herb, erect or decumbent. Rhizome short, 2.7-4.7 mm in diameter. Rosette leaves vaginated at the basal parts, palmatifid with sub-densely white hirsute hairs mainly in lower side, 1.1-1.8 cm in long, 1.6-2.8 cm wide, three lobed, the lobe deep, apex acute-acuminate, base cordate-emarginate, margin dentate with about 14-40 teeth, petiole 2.5-3.5 cm

long with densely long white hirsute hairs. Lowest cauline leaves similar to rosette, middle and upper cauline leaves bract-like, pedately 3 parted. Inflorescence in a terminal cyme, 9-26 cm height, pubescent, pedicell 2.0-3.5 mm long. Sepals 5, navicular, membranous, appressed white hairs, 4.0-6.5 mm long, 1.5-2.5 mm wide. Petals 5, glossy-yellow, obtuse at tips, sometimes lobed, 5.3-8.0 mm long, 3.8-5.4 mm wide, nectary scale free, flabellate 1-1.5 mm long. Stamens 32-49, filaments 1.5-2.0 mm long, flattened, anthers 1.0-1.2 mm long, about 0.6-0.8 mm wide. Carpels about 14-33 spirally arranged, beaked at tip. Receptacles conical with gynocial tapering gradually from the base, about 1 mm long at fruit. Achenes 12-25 in a head, broadly ovate to sub-orbicular, laterally compressed, smooth, beak distinct, short, slightly recurved, 2.0-3.1 mm long, 1.5-2.2 mm wide. Flowering in early May-middle August. $2n=14$.

Japanese name: Ko-Kinpouge.

Other specimens examined: Shikoku Isl., Tokushima Pref., Miyoshi-gun, Higashi-Iyayama-son (village), Izari-pass, August 11, 1973, *C. Abe 47527* (TKPM); Shikoku Isl., Tokushima Pref., Miyoshi-gun, Higashi-Iyayama-son (village), Izari-pass, August 3, 1956, *C. Abe 12001* (TKPM).

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